
Genetic Study of Five Populations of Bihar, India

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Abstract Four-hundred fifty-nine people, including 106 Santals, 43 Bhuiyas, 107 Sakaldipi Brahmins, 108 Chamars, and 95 Ansari Muslims, of the Giridhi district of Bihar have been tested for transferrin, group-specific component, phosphoglucomutase subtypes, and glyoxalase-I, 6-phosphogluconate dehydrogenase, and adenylate kinase types. Genetic distance estimates by both dendrogram and principal component methods for these 5 populations and the Oraons on the basis of 19 alleles at 6 polymorphic loci indicate 2 major clusters: Brahmins and Muslims, the latter of which is composed of two subclusters (Santals and Bhuiyas, and Oraons and Chamars). The Santal and Bhuiya tribes both speak Mundari, whereas the Oraons speak a Dravidian language. The Chamars, although low-caste Hindus, seem to have a non-Europoid origin, as do the Oraons.

Many diverse endogamous groups based on caste hierarchy, language, and religion in India provide unique opportunities for genetic and anthropologic study. Furthermore, throughout the long political history of the Indian subcontinent, the land has been invaded and ruled by many external forces of varied ethnic origins. The state of Bihar is bounded on the north by Nepal, on the east by West Bengal, on the south by Orissa, and on the west by Madhya Pradesh and Uttar Pradesh. Since the early Vedic period (beginning 1500 B.C.), several kingdoms have existed in Bihar. Northern Bihar was known as Magadha in ancient times. In those days the capital of the Magadha empire was located in Pataliputra (modern state capital, Patna) from where two great dynasties, the Maurya (400–300 B.C.) and the Gupta (A.D. 400–500), ruled India. The population of the modern state of Bihar, numbering about 50 million, includes different religious and tribal groups. Eighty-five percent of the Bihar population is Hindu, and the rest are Muslims and tribals. Al-

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though only 9% of the population of Bihar is represented by tribals, 2 of the 5 most populous tribes of the country, with a population of more than 3 million each, live in this state. They are the Santals and the Oraons. In addition, there are many smaller tribes.

The majority of the Bihar population, however, is formed from different caste groups of Hindus and a sizable Muslim population. Thus the population of Bihar provides an interesting context in which to study genetic heterogeneity.

Only limited work on blood genetic markers has been carried out in Bihar. The most widely studied population is that of the Oraon tribe (Sarkar 1937; Kirk et al. 1962; Mukherjee et al. 1975; Saha et al. 1988). Some studies on a limited number of blood genetic markers have been reported in the Santal tribes of Bihar and West Bengal (Sarkar 1937; Sarkar and Sen 1952; Choudhury et al. 1967; Das et al. 1977; Mukherjee et al. 1977; Giri et al. 1982; Reddy et al. 1983). The populations of the neighboring states of West Bengal and to some extent Orissa, have been studied more systematically, however (Chakraborty et al. 1986; Papiha et al. 1988).

In the present investigation we report the distribution of serum transferrin (TF), group-specific component (GC), and red-cell phosphoglucosmutase (PGM) subtypes and three red-cell enzyme types in five populations of Bihar belonging to two major tribes (Santal and Bhuiya), two Hindu caste groups (Sakaldipi Brahmin and Chamar), and one Muslim (Ansari) population. The objectives of the present study are (1) to examine the genetic heterogeneity in the populations of Bihar and (2) to estimate the genetic relationships among different populations of the state by genetic distance analyses.

Materials and Methods

Four-hundred fifty-nine blood samples were collected from members of five populations of the Giridhi district of Bihar. The district is a part of the Chotanagpur plateau. Ethnohistoric sketches of the five populations are presented in what follows.

Santals. The Santals are the most widely distributed and the largest Mundari-speaking tribe in eastern India. They number over 3 million people, distributed mainly in Bihar, neighboring West Bengal, and Orissa. They are settled agriculturists now, although until recently they were primarily forest dwellers engaged in shifting cultivation, food gathering, hunting, and fishing. They worship the Sun God and the Mountain God. Each family has its own family deity.

Bhuiyas. The Bhuiyas, numbering a little over 500,000, are widespread in Bihar and northern Orissa. Some of them are now Hindu. *Bhuiya* means landholders. Today they are mostly engaged as mine workers and casual laborers, although in the past they were mainly cultivators. Although they have a distinct language of their own, they are referred to as an offshoot of the Munda tribe (Risley 1891). They have youth dormitories.

Sakaldipi Brahmins. Brahmins in southern Bihar observe a peculiar custom similar to that of the Saraswat Brahmins of Punjab: Unlike other Brahmins a Sakaldipi can marry within his *gotra* (Bhattacharya 1896). There are a few pandits, landholders, and teachers among them, but the majority minister to the other castes as priests.

Chamars. The Chamars, a large group, are the most widespread low caste Hindu population in India. The word *chamar* is derived from *chamakar*, a worker engaged in leatherwork. Risley (1891) thought, however, that the Chamars were a caste engaged in filthy and menial occupation and must have been recruited from the non-Aryan races. In Bihar the Chamars practice consanguineous marriage.

Ansari Muslims. There are many sects of Muslims and one of them is Ansari. In the Giridhi district the Ansari Muslims are large in number and engage in trade, white-collar jobs, and to some extent agriculture. They also practice consanguinity.

Only unrelated individuals were selected at random for the study. Approximately 1 ml of blood was collected in EDTA tubes from a finger prick. Plasma and cells were separated and stored at -20°C in Calcutta. Samples were brought to Singapore in wet ice in a thermoflask. Transferrin and group-specific component subtypes were determined by isoelectric focusing, following essentially the same method described by Saha (1987a, 1989). Red-cell phosphoglucomutase subtypes were typed by starch-gel electrophoresis and isoelectric focusing (Saha 1987b). Red-cell glyoxalase-I (GLO), 6-phosphogluconate dehydrogenase (PGD), and adenylate kinase (AK) were typed by starch-gel electrophoresis (Saha et al. 1988).

Gene frequencies were calculated by the gene counting method. Hardy-Weinberg equilibrium was estimated by the chi-square (χ^2) test. The genetic relationships among the five populations and the Oraons of Bihar were studied by standard genetic distance analysis (Nei 1972; Nei and Roychoudhury 1974). The dendrogram was derived from the distance matrix by using cluster and tree procedures employing Ward's minimum variance method (Ward 1963). In addition, principal component

analysis was also performed from the distance matrix using the SAS computer program. All the computations were carried out on a main-frame IBM 3081.

Results and Discussion

Table 1 shows the distribution of transferrin, group-specific component, and phosphoglucomatase-I subtypes in five populations of Bihar.

Transferrin. The Brahmins and Muslims had a higher frequency of *TF*CI* than the Santals, Bhuiyas, and Chamars. A similar low frequency of *TF*CI* has been reported for the Oraons and some other tribes in India (Saha et al. 1988; Walter et al. 1981, 1983; Mukherjee et al. 1987). Caste Hindus have a slightly higher frequency of *TF*CI*.

*TF*DChi* was present in low frequency in the Santals (0.02). A much higher frequency (0.07) of the allele has been reported in the Oraons of Bihar. *TF*DChi* has been reported in low frequency (0.01–0.04) in many other tribes of India. Caste Hindus usually lack *TF*DChi* (Papiha et al. 1988).

*TF*C3* was also observed only in the Santals. A low frequency of *TF*C3* has been observed in some Indian populations.

Group-Specific Component. The frequency of *GC*2* was found to be lowest in the Muslims (0.17) and highest in the Bhuiyas (0.39). The Santals had a frequency of 0.29, and the frequency was 0.23 in the Brahmins and 0.32 in the Chamars. The Oraons of Bihar had a *GC*2* frequency of 0.26, which is similar to that in the Santals of the present series (Saha et al. 1988). The Muslims of India and Bangladesh have been reported to have a higher frequency of *GC*2* than in the present series of Ansari Muslims (Papiha et al. 1987; Saha 1987a). A wide range of *GC*2* frequencies has been observed in both the tribal and caste populations of India (Papiha et al. 1987; Walter et al. 1984).

The frequency of *GC*IF* was found to be 0.29 in the Santals, 0.39 in the Bhuiya, and 0.32 in the Chamar. It was much lower in the Brahmins (0.23) and Muslims (0.17). A similar low frequency of *GC*IF* has been observed in five Muslim populations by Papiha et al. (1987). The Oraons have a much higher *GC*IF* (0.30–0.38). A wide range of *GC*IF* frequencies has been reported in the Indian population by Walter et al. (1984) and Papiha et al. (1987) without a definite pattern. In general, Mongoloid populations have a higher frequency of *GC*IF* (Saha 1989).

Phosphoglucomutase 1. The frequency of *PGM1*1* was found to be 0.70 in the Santals and Brahmins and 0.64 in the Bhuiyas and Muslims.

Table 1. Distribution of Transferrin, Group-Specific Component, and Phosphoglucomutase (Locus 1) Subtypes in Five Populations of Bihar, India

Component	Frequency				
	Santals	Bhuiyas	Brahmins	Chamars	Muslims
Transferrin					
Phenotype					
TF C1	54	18	76	58	62
TF C1,C2	40	22	26	33	27
TF C2	7	3	5	17	6
TF C1,C3	1	–	–	–	–
TF C1,D	3	–	–	–	–
TF C2,D	1	–	–	–	–
Total	106	43	107	108*	95
Allele					
TF*C1	0.717	0.674	0.832	0.690	0.795
TF*C2	0.259	0.326	0.168	0.310	0.205
TF*C3	0.005	–	–	–	–
TF*DChi	0.019	–	–	–	–
Group-specific component					
Phenotype					
GC 1F	9	7	7	12	3
GC 1F,1S	25	10	19	21	13
GC 1S	20	9	25	18	21
GC 1F,2	19	10	15	23	13
GC 1S,2	28	6	29	22	31
GC 2	6	2	10	11	15
Total	107	44	105	107	96
Allele					
GC*1F	0.290	0.386	0.229	0.318	0.167
GC*1S	0.435	0.386	0.467	0.369	0.448
GC*2	0.276	0.227	0.305	0.313	0.385
Phosphoglucomutase locus 1					
Phenotype					
PGM1 1A	34	10	22	35	30
PGM1 1A,1B	3	2	5	5	6
PGM1 1B	2	0	2	2	1
PGM1 1A,2A	17	7	22	11	24
PGM1 1A,2B	0	0	4	3	5
PGM1 1B,2A	2	1	0	2	0
PGM1 1B,2B	0	0	0	1	4
PGM1 2A	8	4	4	4	8
PGM1 2A,2B	1	0	1	0	4
PGM1 2B	2	1	0	0	2
Other	0	0	0	2	1
Total	69	25	60	65	85
Allele					
PGM1*1A	0.638	0.580	0.625	0.692	0.565
PGM1*1B	0.065	0.060	0.075	0.092	0.071
PGM1*2A	0.261	0.320	0.258	0.161	0.259
PGM1*2B	0.036	0.040	0.042	0.031	0.100
PGM1*6	–	–	–	–	0.006
PGM1*7	–	–	–	0.023	–

a. $\chi^2_3 = 8.81$.

The Chamars had the highest frequency, 0.78. The frequency of *PGM1*1* ranges among Indian populations from 0.44 to 0.79. The Oraons of Bihar had a frequency of 0.70, and the Konds of Orissa had a frequency of 0.78. In general, the caste populations have a higher frequency of *PGM1*1* than the tribals.

The Chamars and Muslims have *PGM1*7* and *PGM1*6*, respectively, although in low frequencies. *PGM1*6* has been reported in some other tribal populations of India (Saha 1988).

The frequency of *PGM1*1A* was found to be 0.69 in the Chamars and 0.63 in the Santals and Brahmins, whereas it was 0.62 in the Oraons of Bihar. The Muslims of the present series had a frequency of 0.56, and the Bengali Muslims had a slightly higher frequency (0.60). The frequency of *PGM1*1B* was almost the same in all five populations and the Oraons (0.06–0.09). The frequency of *PGM1*2A* varied from 0.16 in the Chamars to 0.32 in the Bhuiyas. A similar range of *PGM1* subtype allelic frequencies has been reported in the Indian population. The frequency of *PGM1*2B* varied from 0.04 in the Santals to 0.10 in the Muslims. However, *PGM1*2B* was very low in the Bengali Muslims (0.03).

Red Cell Enzyme Types. Table 2 shows the distribution of three red cell enzymes in five populations of Bihar.

Glyoxalase-I. The Brahmins and Chamars have a slightly higher frequency of *GLO*1* (0.27) than the other 3 populations (0.23). The Oraons have a much higher *GLO*1* frequency (0.35–0.36) (Saha et al. 1988). Koya Dora and Konda tribes and the Mundas have similarly low frequencies of *GLO*1* (Reddy et al. 1983). The frequency of *GLO*1* in different populations of India varies from 0.15 to 0.36.

6-Phosphogluconate Dehydrogenase. The Santals completely lack *PGD*C*, whereas the other populations have frequencies ranging from 0.01 in the Muslims to 0.04 in the Bhuiya. In general, the frequency of *PGD*C* is low in the Indian population except among the Lepchas, Khasis, and Nagas of the highlands and the Nepalese and Bhutanese (Saha et al. 1987; Saha and Tay 1990). In the highlanders *GD*Q0* is also low in frequency.

This reciprocal relationship between *PGD*C* and *GD*Q0* may be due to relaxation of malarial selection pressure at high altitude and institution of hypoxia-mediated selective pressure.

Adenylate Kinase. The Santals and Bhuiyas have *AK*2* frequencies of 0.16 and 0.09, respectively; the frequency was low in the other populations studied. The Oraons also have a higher frequency of *AK*2* (0.09–0.10). The Mongoloids generally have lower frequencies of *AK*2*. The

Table 2. Distribution of Three Red Cell Enzyme Polymorphisms in Five Populations of Bihar, India

Enzyme	Frequency				
	Santals	Bhuiyas	Brahmins	Chamars	Muslims
Glyoxalase I					
Phenotype					
GLO 1	2	1	5	7	12
GLO 1,2	30	8	38	26	17
GLO 2	41	13	45	42	60
Total	73	22	88	75	89 ^a
Allele					
GLO*1	0.233	0.227	0.273	0.267	0.230
GLO*2	0.767	0.773	0.727	0.733	0.770
6-Phosphogluconate-dehydrogenase					
Phenotype					
PGD A	86	21	92	81	79
PGD A,C	0	2	4	4	2
Total	86	23	96	85	81
Allele					
PGD*A	1.000	0.957	0.979	0.977	0.988
PGD*C	0.000	0.043	0.021	0.023	0.012
Adenylate kinase					
Phenotype					
AK 1	59	14	92	81	79
AK 1,2	27	3	4	4	2
Total	85	17	96	85	81
Allele					
AK*1	0.841	0.912	0.979	0.977	0.988
AK*2	0.159	0.088	0.021	0.023	0.012

a. $\chi^2_3 = 18.6$.

Nilgiri tribes also have higher AK*2, ranging from 0.06 to 0.15. It is possible that the AK*2 gene found in Indian populations spread from tribal populations (Saha et al. 1976).

Hardy-Weinberg Equilibrium. The phenotypic distribution of all the loci in all the populations was at Hardy-Weinberg equilibrium except for TF in the Chamars ($\chi^2_3 = 8.81$) and GLO 1 in the Muslims ($\chi^2_3 = 18.55$). The excess of homozygosity at the TF locus could be explained by the practice of consanguinity among the Chamars. In both instances there was an excess of homozygosity. This may be due to chance observation, because many estimates have been made on the small sample sizes.

Average Heterozygosity. The average heterozygosity (Table 3) was lowest in the Brahmins and Muslims (0.32 ± 0.10 and 0.32 ± 0.11 , respectively), whereas it ranged from 0.34 ± 0.10 to 0.39 ± 0.10 in the

Table 3. Standard Genetic Distance Matrix of Six Populations of Bihar, Eastern India, Based on 19 Alleles at 6 Polymorphic Loci^a

<i>Population</i>	<i>Santal</i>	<i>Bhuiya</i>	<i>Brahmin</i>	<i>Chamar</i>	<i>Muslim</i>	<i>Oraon</i>
Santal	0.3689 ± 0.0914					
Bhuiya	0.0055 ± 0.0021	0.3736 ± 0.0902				
Brahmin	0.0083 ± 0.0033	0.0131 ± 0.0072	0.3218 ± 0.1016			
Chamar	0.0081 ± 0.0036	0.0081 ± 0.0053	0.0090 ± 0.0054	0.3438 ± 0.1014		
Muslim	0.0109 ± 0.0050	0.0157 ± 0.0109	0.0030 ± 0.0017	0.0113 ± 0.0066	0.3249 ± 0.1074	
Oraon	0.0092 ± 0.0044	0.0073 ± 0.0045	0.0135 ± 0.0073	0.0061 ± 0.0023	0.0188 ± 0.0105	0.3895 ± 0.1009

a. Figures on the diagonal are the average heterozygosities per locus.

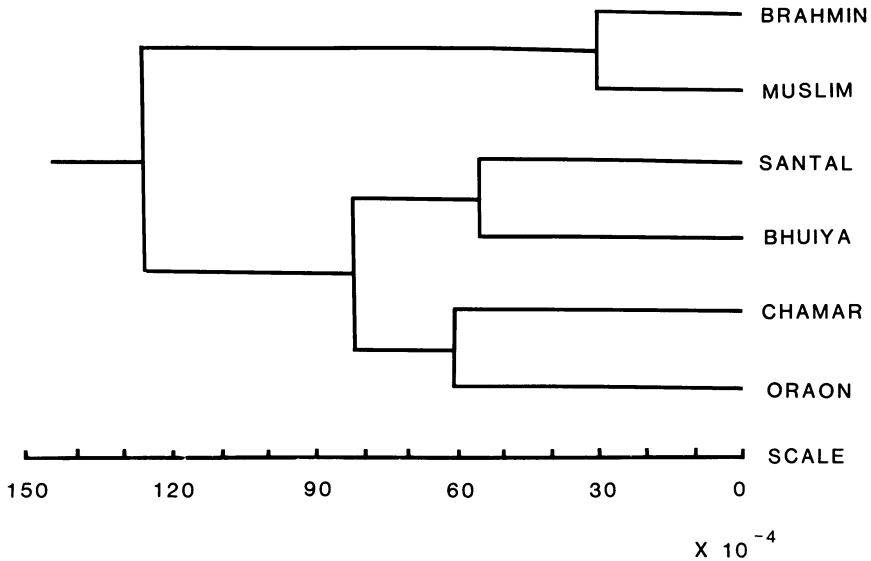


Figure 1. Dendrogram showing genetic relationship of 6 populations of Bihar, India, based on 6 polymorphic loci controlling 19 alleles.

other populations. The degree of variability, as judged by average heterozygosity, was not much different in these populations.

Genetic Distances. The genetic distance matrix of the 5 populations and the Oraons on the basis of 19 alleles at 6 polymorphic loci is presented in Table 3. The dendrogram appears in Figure 1, and the resulting eigenvector based on the first and third principal components is presented in Figure 2. The clustering of the populations in the eigenvector is the same as in the dendrogram. The Brahmins and the Chamars are from high- and low-caste Hindus, although the dendrograms indicate that they are genetically quite distant, with the Chamars clustering with the Oraons. The Muslims cluster with the Brahmins. This may be because the Muslims are recent converts from the caste Hindus. Risley (1891) proposed a non-Aryan (non-Europoid) origin for the Chamars. The clustering of the Chamars with the Oraons, distant from the Brahmins, fits well with Risley's hypothesis. Usually low-caste Hindus, such as the Chamars, resisted conversion to Islam. However, it is not possible to draw any final conclusions because only one of the higher caste groups has been investigated here.

The most interesting aspect of the study is the clustering of the Santals and Bhuiyas together in a separate cluster from the Oraons. This is also expected from the ethnohistoric and linguistic accounts of these

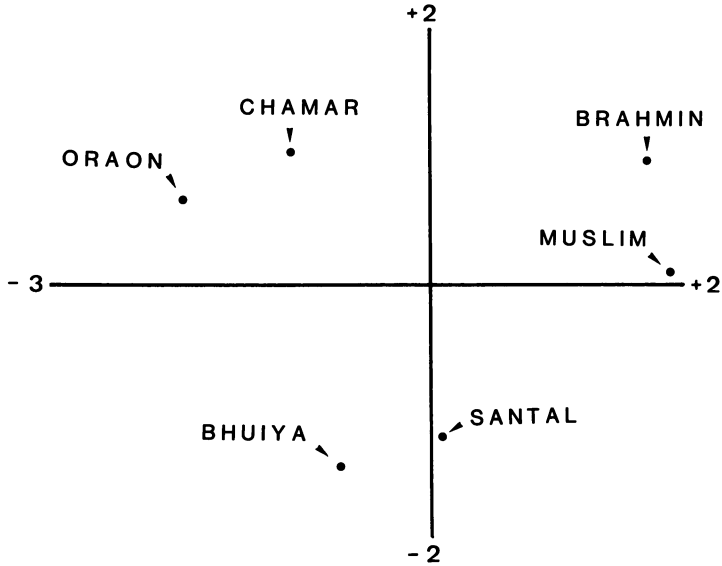


Figure 2. Eigenvector of first and third principal components showing genetic relationship of 6 populations of Bihar, India, based on 6 polymorphic loci controlling 19 alleles.

three tribes. The Santal and Bhuiya tribes speak Mundari, whereas the Oraons speak the Kurukh dialect of the Dravidian language. They have been described as proto-Australoid stock, and it is believed that, although they now reside on the Chota Nagpur plateau of Bihar, they came from southern India and settled in their present location several centuries ago.

Although some significant evidence in support of the ethnohistoric account of the populations has emerged from this study, it should be mentioned that only a limited number of polymorphic systems have been studied and in some cases the sample size was not adequate (e.g., for the Bhuiyas).

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